ORIGINAL ARTICLE

Serum concentration of selected biochemical markers of endothelial dysfunction and inflammation in patients with the varying activity of inflammatory bowel disease

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KEY WORDS

endothelium, inflammatory bowel disease, microparticles, monocyte chemoattractant protein 1, soluble CD40 ligand

ABSTRACT

INTRODUCTION Endothelial dysfunction leads to an increased expression of cell adhesion molecules, leukocyte diapedesis, vascular smooth-muscle tone, excessive permeability of vascular walls, and increased procoagulant activity.

OBJECTIVES We investigated whether serum levels of several endothelial and platelet activation markers correlated with disease activity in patients with inflammatory bowel disease (IBD).

PATIENTS AND METHODS This study included 56 patients with ulcerative colitis, 66 with Crohn disease, and 40 healthy controls. We measured the complete blood count and levels of fibrinogen, C-reactive protein, albumin, interleukin 6, tumor necrosis factor α, E-selectin, P-selectin, monocyte chemoattractant protein 1 (MCP-1), soluble CD40 ligand (sCD40L), and microparticles.

RESULTS There were no significant differences in the median levels of E-selectin, P-selectin, MCP-1, sCD40L, and microparticles between patients with active IBD, those with inactive IBD, and healthy controls. The clinical disease activity assessed with the Mayo scale in the ulcerative-colitis group was weakly, positively correlated with sCD40L ($R = 0.32$, $P = 0.02$), P-selectin ($R = 0.32$, $P = 0.02$), and inflammatory marker levels. The clinical disease activity index in the Crohn disease group was positively correlated with the markers of inflammation yet not with the markers of endothelial activity.

CONCLUSIONS E-selectin, P-selectin, sCD40L, MCP-1, and microparticle levels do not significantly differ between patients with the varying activity of IBD. However, due to the observed correlations, further studies of a larger patient group should be conducted to confirm our observations.

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INTRODUCTION The vascular endothelium, the largest endocrine organ of the human body, plays a pivotal role in numerous physiological processes, such as coagulation and fibrinolysis, cell proliferation, angiogenesis, regulation of substance transport, leukocyte migration across vascular walls, platelet interaction, and regulation of vascular tone.[1-3] Endothelial dysfunction is an imbalance between vasodilating and vasoconstricting substances produced by (or affecting) endothelial cells.[4,5] It is characterized by a decreased bioavailability of nitric oxide due to an increased production of reactive oxygen species.[4-6] Free radicals, which are generated at the site of inflammation and injury,[7] can disrupt the balance of nitric oxide and damage the endothelium.[4]

Chronic inflammatory processes cause functional and structural changes in the vascular endothelium and affect its activation.[2] Endothelial dysfunction leads to an increased expression of cell adhesion molecules, leukocyte diapedesis, vascular smooth-muscle tone, excessive permeability of vascular walls, and increased procoagulant activity.[2] Endothelial dysfunction is

WHAT'S NEW?

The intestinal vascular microcirculation plays a relevant role in the initiation and maintenance of the inflammatory process. It regulates the recruitment of leukocytes from the vascular circulation into inflamed tissues and expresses cell adhesion molecules and chemokines that enhance interactions with leukocytes. Endothelial dysfunction is observed in various diseases, including inflammatory bowel disease (IBD). To assess endothelial cell activity, biochemical methods such as the release of substances from a normal or damaged endothelium can be used. We investigated serum levels of selected endothelial activation markers in patients with a varying activity of IBD. Our study did not show any significant differences in levels of E-selectin, P-selectin, monocyte chemoattractant protein 1, soluble CD40 ligand, and microparticles in patients with active IBD, those with inactive IBD, and healthy controls. However, further studies including large samples are needed to confirm these observations.

involved in various diseases, including peripheral vascular disease, stroke, venous thrombosis, diabetes, insulin resistance, hypercholesterolemia, cardiac disease, tumor growth and metastasis, chronic kidney failure, rheumatoid arthritis, viral infections, and inflammatory bowel disease (IBD).[2,4,7-10]

The multifactorial etiology of IBD requires further elucidation. Genetically predisposed patients with ulcerative colitis and Crohn disease show changes in gut microbiota, disruption of epithelial barrier function, and chronic immune activation.[7,10] In addition, oxidative stress causes gastrointestinal tract injury.[1,6,8]

Physical and biochemical methods can be used to assess endothelial cell activity, such as the release of substances from a normal or damaged endothelium.[2] Cellular or chemical biomarkers comprise factors released by endothelial cells, such as cell adhesion molecules, vascular endothelial growth factor, von Willebrand factor, tissue plasminogen activator, thrombomodulin, asymmetric dimethylarginine, disintegrin and metalloproteinase with thrombospondin motif 13, and angiopoietins[5,7-10] or recently discovered microparticles, vesicles released by endothelial cells, platelets, erythrocytes, monocytes, and lymphocytes (from their cell membranes).[10]

This study compared the serum levels of endothelial dysfunction biomarkers (E-selectin and P-selectin), monocyte chemoattractant protein 1 (MCP-1), and soluble CD40 ligand (sCD40L), as well as microparticle activity, between patients with IBD and healthy controls. We investigated the potential association of these markers with clinical disease activity in patients with IBD.

PATIENTS AND METHODS

Study population

Study participants were recruited from the Department of Gastroenterology and Hepatology of the University Hospital in Kraków, Poland. All of them signed a written informed consent form, and the study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the Bioethics Committee at Jagiellonian University in Kraków, Poland (KBET/54/B/2012).

The study prospectively enrolled 56 patients with ulcerative colitis (median age [interquartile range (IQR)], 37 [26–51.25] years; 28 men) and 66 patients with Crohn disease (median [IQR] age, 31 [26–37] years; 33 men). Inflammatory bowel disease was diagnosed using clinical, radiological, endoscopic, and histopathological criteria.[11] Based on 2 scores, the study patients were divided into subgroups with inactive Crohn disease (Crohn disease activity index [CDAI] <150) and active Crohn disease (CDAI ≥150); and inactive ulcerative colitis (Mayo scale score <4) and active ulcerative colitis (Mayo scale score ≥4).[12,13] The control group included 40 healthy volunteers (median [IQR] age, 32 [25–45] years).

The exclusion criteria were as follows: any known acute or chronic infection, pregnancy or lactation, autoimmune diseases, rheumatoid arthritis, hypertension, coronary artery disease, chronic heart failure, diabetes, dyslipidemia, chronic kidney disease, severe respiratory failure, peripheral vascular disease, stroke, venous thrombosis, hematologic disorders, or malignancies.

Methods

Patient demographics, clinical characteristics, results of gastrointestinal tract examination, IBD history, and treatments were recorded and the body mass index (BMI) was calculated. Blood samples were collected after an overnight fast and on the same day. A complete blood count, a lipid profile, and C-reactive protein (CRP), albumin, alanine aminotransferase, creatinine, glucose, and fibrinogen levels were measured in the hospital laboratory using standard procedures.

Serum was obtained from another blood sample, which was later used to determine interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), E-selectin, P-selectin, MCP-1, sCD40L, and microparticle levels.

An enzyme-linked immunosorbent assay (ELISA) was used to measure IL-6 (IL-6 HS, R&D Systems, Minneapolis, Minnesota, United States) and TNF-α levels (TNF HS, R&D Systems).

P-selectin, E-selectin, sCD40L, and MCP-1 serum levels were determined using an ELISA according to the manufacturer's instructions (Human CCL2 / MCP-1 Quantikine ELISA kit, Human CD40 ligand / TNFSF5 Quantikine ELISA kit, Human P-selectin / CD62P Quantikine ELISA kit, Human sE-selectin / CD62E Quantikine ELISA kit; R&D Systems). Optical density was measured on a plate reader (450-nm wavelength), and data were collected using the Gen 5 software (BioTek, Winooski, Vermont, United States). A 4-parameter curve fit was used to generate the standard curve.

Microparticle procoagulant activity in human serum was determined using an ELISA according to the manufacturer's instructions (Zymuphen MP-Activity, Hyphen BioMed, Neuville-sur-Oise, France). Optical density was measured on a plate

TABLE 1 Patient characteristics

Characteristic		Ulcerative colitis (n = 56)	Crohn disease (n = 66)	Controls (n = 40)	*P* value
Male sex, n (%)		28 (50)	33 (50)	20 (50)	0.99
Age, y, median (IQR)		37 (26–51.2)	31 (26–37)	32 (25–45)	0.06
BMI, kg/m², median (IQR)		22.7 (19.6–25.1)	21.5 (18.7–24.4)	23.4 (20.8–26)	0.08
Smoking status, n (%)		8 (14.3)	17 (25.8)	10 (25)	0.23
Disease duration, y, median (IQR)		6 (2–11)	4 (1–9)	–	0.14
Disease location, n (%)	E1 (proctitis)	5 (8.9)	–	–	–
	E2 (left-sided)	28 (50)	–	–	–
	E3 (extensive)	23 (41.1)	–	–	–
	L1 (ileum)	–	13 (19.7)	–	–
	L2 (colon)	–	10 (15.2)	–	–
	L3 (ileocolon)	–	43 (65.1)	–	–

Abbreviations: BMI, body mass index; IQR, interquartile range

reader (450-nm wavelength), and data were collected using the Gen 5 software (BioTek). Linear curve fitting was used to generate the standard curve.

Statistical analysis Statistical analyses were performed using the Statistica software, version 12.0 (StatSoft, Inc., Tulsa, Oklahoma, United States). Data were expressed as percentage (categorical variables), mean (SD) (normally distributed variables), and median and interquartile range (nonnormally distributed continuous variables). The Shapiro–Wilk test was used to assess the normality of data distribution. The analysis of variance and the Kruskal–Wallis test were used to compare continuous variables. The Tukey test or the Dunn–Bonferroni approach were applied for pairwise comparison for post hoc testing after the analysis of variance and the Kruskal–Wallis test, respectively. Categorical variables were analyzed using the χ^2 test. The associations among clinical characteristics, IBD activity, and biochemical parameters were evaluated by calculating the Spearman rank correlation coefficient. A *P* value less than 0.05 was considered significant.

RESULTS

Patient characteristics Detailed patient characteristics are presented in TABLE 1. No significant differences in sex, age, BMI, or smoking habits were detected between the study groups. All patients with ulcerative colitis received mesalamine (2–4 g/d), 12 patients (21%) were on maintenance therapy with thiopurines, 17 (30%) received glucocorticoids, and 2 (3.6%) received biological treatment with infliximab. Among patients with Crohn disease, those with disease location at L2 and L3 received mesalamine (2–4 g/d), 30 individuals (45%) were on maintenance immunosuppressive therapy, 23 (35%) received glucocorticoids, and 4 (6%) received biological treatment with infliximab or adalimumab. None of the study patients had undergone surgery for ulcerative colitis. However, 31 patients (47%) with Crohn disease had at least 1 surgery due to disease complications. None of them had stoma. Two patients underwent right hemicolectomy.

Among the study patients with ulcerative colitis, disease remission was observed in 19 (34%) individuals, mild disease exacerbation in 17 (26%), moderate exacerbation in 18 (32%), and severe exacerbation in 2 (3.6%). Among those with Crohn disease, 33 (50%) were in remission, 12 (18%) had mild disease exacerbation, 21 (32%) moderate exacerbation, and none of the patients experienced severe exacerbation.

Laboratory test results Patients with active IBD had significantly higher levels of inflammatory markers (white blood cells [WBCs], CRP, fibrinogen, and IL-6) compared with those with inactive disease and controls (TABLES 2 and 3).

The active and inactive subgroups of patients with ulcerative colitis and Crohn disease showed no differences in microparticle activity and levels of selectins, sCD40L, or MCP-1 (TABLES 2 and 3). In the ulcerative colitis group, no differences were found in the levels of the analyzed markers depending on various stages of the disease (TABLE 4). Patients with severe flare were excluded from this analysis because of the limited number of subjects.

Patients with inactive Crohn disease, those at various stages of active Crohn disease, and controls showed no difference in microparticle activity or levels of selectins or sCD40L (TABLE 5). Patients with moderate Crohn disease had a higher median (IQR) level of MCP-1 than those with mild disease (144.01 [87.59–221.84] vs 94.81 [85.23–122.2] pg/ml; *P* = 0.04). The study did not reveal any differences in patients with Crohn disease with or without a history of surgery: microparticles (median [IQR], 23.68 [11.72–34.78] vs 17.23 [11.79–27.19] nM; *P* = 0.33), sCD40L (206.61 [147.3–719.69] vs 318.26 [178.84–776.27] pg/ml; *P* = 0.41), MCP-1 (149.96 [91.79–210.9] vs 125.98 [86.5–188.06] pg/ml; *P* = 0.25), E-selectin (27.25 [20.25–35.19] vs 26.8 [17.53–36.46] ng/ml; *P* = 0.69), P-selectin (40 [34.08–53.06] vs 39.08 [31.76–47.08] ng/ml; *P* = 0.33).

TABLE 2 Endothelial activation and inflammatory markers in patients with active or inactive ulcerative colitis and healthy controls

Parameter	Inactive ulcerative colitis (n = 19)	Active ulcerative colitis (n = 37)	Controls (n = 40)	*P* value
Microparticles, nM	14.78 (6.29–35.6)	24.4 (14.49–44.47)	30.04 (17.32–42.88)	0.07
sCD40L, pg/ml	196.83 (130.25–414.07)	353.8 (155.45–543.93)	295.92 (121.4–694.98)	0.05
MCP-1, pg/ml	121.9 (87.34–168.35)	129.44 (105.02–173.79)	149.06 (106.2–229.24)	0.19
E-selectin, ng/ml	21.34 (18.51–32.06)	25.37 (19.19–29.87)	25.87 (17.52–32.34)	0.83
P-selectin, ng/ml	36.36 (28.56–49.38)	40.26 (33.66–53.22)	43.29 (37.35–54.7)	0.08
Albumin, g/l	45 (44–47)	41[a,b] (35–44)	47 (43–48)	$<$0.001
CRP, mg/l	0.99 (0.71–1.72)	8.63[a,b] (4.02–28)	0.94 (0.47–1.39)	$<$0.001
Fibrinogen, g/l	2.56 (2.25–3.43)	4.32[a,b] (3.55–5.9)	2.8 (2.45–3.1)	$<$0.001
IL-6, pg/ml	1.37 (0.88–2.46)	3.36[a,b] (1.66–6.65)	1.43 (0.94–2.13)	0.001
PLT, $\times 10^3/\mu l$	235 (205–295)	270[b] (231–359)	224 (185–252)	0.01
TNF-α, pg/ml	1.23 (1.02–3.37)	1.89[a] (1.38–3.05)	1.66 (1.11–3.23)	0.39
WBC, $\times 10^3/\mu l$	5.82 (4.63–6.8)	7.46[a,b] (6.47–9.92)	5.75 (4.65–7.6)	$<$0.001

Data are presented as median (interquartile range).

a A significant difference was found between the active ulcerative-colitis and inactive ulcerative-colitis groups.

b A significant difference was found between the active ulcerative-colitis and control groups.

Abbreviations: CRP, C-reactive protein; IL-6, interleukin 6; MCP-1, monocyte chemoattractant protein 1; PLT, platelets; sCD40L, soluble CD40 ligand; TNF-α, tumor necrosis factor α; WBC, white blood cell

TABLE 3 Endothelial activation and inflammatory markers in patients with active or inactive Crohn disease and healthy controls

Parameter	Inactive Crohn disease (n = 33)	Active Crohn disease (n = 33)	Controls (n = 40)	*P* value
Microparticles, nM	18.63 (12.94–31.06)	19.41 (11.55–34.71)	30.04 (17.32–42.88)	0.09
sCD40L, pg/ml	225.58 (157.58–501.36)	281.49 (179.77–719.69)	295.92 (121.4–694.98)	0.74
MCP-1, pg/ml	145.43 (100.63–189.27)	129.46 (87.34–210.91)	149.06 (106.2–229.24)	0.19
E-selectin, ng/ml	21.7 (18.45–29.48)	27.92 (23.29–39.17)	25.87 (17.52–32.34)	0.09
P-selectin, ng/ml	39.89 (31.94–46.55)	39.78 (33.36–47.08)	43.29 (37.35–54.7)	0.3
Albumin, g/l	45 (41–47)	37[a,b] (35–42)	47 (43–48)	$<$0.001
CRP, mg/l	1.31 (0.9–3.48)	23.2[a,b] (10.8–44.6)	0.94 (0.47–1.39)	$<$0.001
Fibrinogen, g/l	3.42 (2.68–3.97)	5.38[a,b] (4.76–6.04)	2.8 (2.45–3.1)	$<$0.001
IL-6, pg/ml	1.37 (0.92–3.16)	4.07[a,b] (2.11–6.51)	1.43 (0.94–2.13)	$<$0.001
PLT, $\times 10^3/\mu l$	279 (223–311)	332[a,b] (280–420)	224 (185–252)	$<$0.001
TNF-α, pg/ml	1.48 (0.98–2.2)	1.64 (1.27–2.17)	1.66 (1.11–3.23)	0.63
WBC, $\times 10^3/\mu l$	5.95 (5.47–7.08)	7.36[b] (6.21–9.93)	5.75 (4.65–7.6)	0.006

Data are presented as median (interquartile range).

a A significant difference was found between the active Crohn-disease and inactive Crohn-disease groups.

b A significant difference was found between the active Crohn-disease and control groups.

Abbreviations: see TABLE 2

TABLE 4 Markers of endothelial activation at various activity stages of ulcerative colitis

Parameter	Mayo score, 0–2 (n = 19)	Mayo score, 3–5 (n = 11)	Mayo score, 6–10 (n = 24)	*P* value
Microparticles, nM	14.78 (6.29–35.66)	26.58 (14.48–40.65)	26.77 (14.5–55.86)	0.09
sCD40L, pg/ml	196.83 (130.25–414.07)	135.65 (114.04–386.43)	416.6 (200.15–625.15)	0.07
MCP-1, pg/ml	121.91 (87.34–168.35)	110.86 (101.18–151.13)	133.18 (100.62–183.04)	0.69
E-selectin, ng/ml	21.34 (18.51–32.06)	20.05 (18.65–26.05)	25.76 (21.26–32.64)	0.22
P-selectin, ng/ml	36.36 (28.56–49.38)	33.66 (32–40.28)	43.32 (37.6–55.7)	0.11

Data are presented as median (interquartile range).

Abbreviations: see TABLE 2

TABLE 5 Markers of endothelial activation at various activity stages of Crohn disease

Parameter	CDAI <150 (n = 33)	CDAI, 150–220 (n = 12)	CDAI, 221–450 (n = 21)	*P* value
Microparticles, nM, median (IQR)	18.63 (12–36.94)	18.6 (13.04–27.48)	19.41 (11.55–47.92)	0.97
sCD40L, pg/ml, median (IQR)	225.58 (157.48–501.36)	214.32 (74.22–666.13)	452.78 (179.77–897.06)	0.69
MCP-1, pg/ml, median (IQR)	145.43 (100.63–189.27)	94.81 (85.23–122.2)	144.01 (87.59–221.84)	0.04
E-selectin, ng/ml, mean (SD)	24.47 (8.72)	32.12 (15.64)	31.87 (14.98)	0.06
P-selectin, ng/ml, mean (SD)	41.95 (14.37)	38.27 (13.12)	49.17 (21.17)	0.15

Abbreviations: CDAI, Crohn disease activity index; others, see TABLE 2

TABLE 6 Markers of endothelial activation depending on the treatment used

Parameter	Mesalamine only	Glucocorticoids	Thiopurines	Glucocorticoids and thiopurines	*P* value
Ulcerative colitis					
Patients, n	32	12	6	4	–
MPs, nM	28.4 (14.58–45.26)	14.93 (9.82–40.42)	9.61 (8.49–39.92)	27.15 (15.33–14.77)	0.44
sCD40L, pg/ml	241.83 (129.35–470.3)	543.93 (122.9–1005.39)	226.53 (201.86–414.07)	306.91 (181.53–412.87)	0.66
MCP-1, pg/ml	114.49 (91.74–153.07)	135.17 (82.77–237.7)	144.59 (122.46–178.02)	140.04 (98.78–247.87)	0.53
E-selectin, ng/ml	25.55 (19.31–29.74)	21.58 (19.18–24.45)	32.95 (19.28–34.38)	23.61 (16.84–30.99)	0.33
P-selectin, ng/ml	40.28 (32–53.4)	34.86 (29.02–44.69)	42.32 (38.4–45.26)	40.23 (27.98–61.92)	0.54
Crohn disease					
Patients, n	22	14	17	9	–
Microparticles, nM	23.53 (12.1–44.59)	16.3 (9.72–26.37)	18.62 (16.25–34.78)	15.54 (11.79–23.08)	0.65
sCD40L, pg/ml	437.63 (147.3–964.72)	200.76 (157.58–261.65)	384.64 (206.61–948.86)	210.34 (157.58–452.78)	0.24
MCP-1, pg/ml	160.6 (91.79–210.67)	124.95 (87.34–146.86)	129.46 (95.73–151.64)	134.63 (90.22–188.06)	0.75
E-selectin, ng/ml	21.1 (19.36–28.96)	27.85 (14.32–37.35)	30.04 (24.59–36.46)	28.53 (23.38–36.8)	0.17
P-selectin, ng/ml	39.89 (38.06–58.6)	38.34 (32.46–42.74)	39.78 (33.36–66.26)	35.66 (28.56–40)	0.46

Data are presented as median (interquartile range) unless otherwise indicated.

All patients with ulcerative colitis were on maintenance therapy with mesalamine. Patients with Crohn disease location L2 and L3 received mesalamine.

Abbreviations: see TABLE 2

In patients with ulcerative colitis and those with Crohn disease, no differences were also found in the levels of endothelial dysfunction parameters depending on the type of therapy used (TABLE 6). However, patients with active IBD who were on maintenance immunosuppressive therapy had a lower median microparticle activity compared with those taking mesalamine only (16.13 [9.04–26.58] vs 26.94 [16.81–49.33] nM; $P = 0.01$). Patients receiving biological treatment were excluded from this analysis because of the limited number of subjects.

Correlations between the analyzed parameters In the ulcerative colitis group, disease activity (assessed using the Mayo scale) correlated positively with sCD40L ($R = 0.32$, $P = 0.02$), P-selectin ($R = 0.32$, $P = 0.02$), WBC ($R = 0.52$, $P < 0.001$), platelet ($R = 0.38$, $P < 0.001$), fibrinogen ($R = 0.63$, $P < 0.001$), TNF-α ($R = 0.27$, $P = 0.047$), and IL-6 ($R = 0.47$, $P < 0.001$) levels, and negatively with hemoglobin ($R = -0.32$, $P = 0.01$) and albumin levels ($R = -0.54$, $P < 0.001$). In the ulcerative colitis group, the only markers of endothelial dysfunction that showed a correlation were E-selectin and P-selectin levels ($R = 0.56$, $P < 0.001$).

In the Crohn disease group, although CDAI did not correlate with the analyzed markers, we found a positive correlation with CRP ($R = 0.71$, $P < 0.001$), fibrinogen ($R = 0.61$, $P < 0.001$), WBC ($R = 0.29$, $P = 0.02$), platelet ($R = 0.51$, $P < 0.001$), and IL-6 ($R = 0.35$, $P = 0.004$) levels, yet a negative correlation with hemoglobin ($R = -0.71$, $P < 0.001$) and albumin levels ($R = -0.66$, $P < 0.001$). The microparticle activity positively correlated with sCD40L ($R = 0.27$, $P = 0.025$) and MCP-1 ($R = 0.24$, $P = 0.046$) levels, and negatively, with E-selectin levels ($R = -0.25$, $P = 0.04$). P-selectin levels positively correlated with MCP-1 levels ($R = 0.3$, $P = 0.01$).

In the control group, P-selectin levels positively correlated with microparticle ($R = 0.6$, $P < 0.001$),

TABLE 7 Correlations between endothelial activation and inflammatory markers in patients with ulcerative colitis

Parameter	Microparticles		sCD40L		MCP-1		E-selectin		P-selectin	
	R value	*P* value	*R* value	*P* value	*R* value	*P* value	*R* value	*P* value	*R* value	*P* value
CRP	0.13	0.33	0.14	0.31	0.07	0.64	0.37	0.006	0.36	0.008
WBC	0.36	0.007	0.2	0.15	0.03	0.82	0.29	0.03	0.25	0.07
PLT	0.31	0.02	0.15	0.27	0.11	0.42	0.25	0.07	0.31	0.02
Albumin	–0.1	0.48	–0.39	0.004	–0.2	0.14	–0.24	0.08	–0.28	0.04
Fibrinogen	0.31	0.02	0.17	0.2	0.18	0.19	0.26	0.06	0.34	0.01
TNF-α	0.23	0.08	0.32	0.02	0.35	0.01	0.48	<0.001	0.2	0.14
IL-6	0.15	0.29	0.31	0.02	0.3	0.02	0.42	0.002	0.28	0.04

Abbreviations: see TABLE 2

TABLE 8 Correlations between endothelial activation and inflammatory markers in patients with Crohn disease

Parameter	Microparticles		sCD40L		MCP-1		E-selectin		P-selectin	
	R value	*P* value	*R* value	*P* value	*R* value	*P* value	*R* value	*P* value	*R* value	*P* value
CRP	0.04	0.75	–0.009	0.94	–0.07	0.59	0.28	0.02	0.03	0.79
WBC	–0.03	0.81	0.05	0.72	–0.05	0.67	0.35	0.005	0.33	0.008
PLT	0.15	0.24	0.2	0.12	0.08	0.54	0.06	0.63	0.19	0.13
Albumin	–0.22	0.08	–0.25	0.04	–0.01	0.94	–0.15	0.24	0.007	0.007
Fibrinogen	0.003	0.98	–0.08	0.52	–0.05	0.67	0.25	0.05	0.08	0.55
TNF-α	–0.03	0.85	0.24	0.06	–0.08	0.52	0.47	<0.001	–0.03	0.8
IL-6	0.31	0.01	0.13	0.3	0.14	0.28	0.23	0.052	0.06	0.64

Abbreviations: see TABLE 2

sCD40L ($R = 0.35$, $P = 0.02$), MCP-1 ($R = 0.5$, $P = 0.001$), and E-selectin ($R = 0.46$, $P = 0.003$) levels, and MCP-1 levels positively correlated with microparticle ($R = 0.4$, $P = 0.01$), sCD40L ($R = 0.62$, $P < 0.001$), and E-selectin ($R = 0.32$, $P = 0.04$) levels.

Correlations between the markers of endothelial dysfunction and inflammation in the ulcerative colitis and Crohn disease groups are presented in TABLES 7 and 8, respectively.

DISCUSSION This study revealed that patients with inactive and active IBD and controls did not differ significantly regarding P-selectin, E-selectin, sCD40L, MCP-1, or microparticle levels. Moreover, in contrast to healthy controls, the selected markers in patients with IBD showed no (or only weak or very weak) correlations with each other, except for P-selectin and E-selectin levels in patients with ulcerative colitis.

Patients with ulcerative colitis and Crohn disease have disrupted immune systems[14] and increased permeability of the epithelial barrier.[15] Thus, antigens are exposed to granulocytes, lymphocytes, and dendritic cells through the damaged submucosa.[14] Tissue-damaging proteases, proinflammatory cytokines, and vasoactive peptides are released from neutrophils and mast cells, causing mucositis.[16] Activated T cells demonstrate delayed apoptosis and persist in the mucosa, contributing to chronic inflammation.[14,16] Furthermore, activated platelets can modulate the activity of endothelial cells.[17]

Although several studies have assessed various markers of endothelial activity in patients with IBD, the results are inconclusive. Conflicting with some studies, our results showed no differences in serum microparticle activity or serum levels of MCP-1, sCD40L, E-selectin, and P-selectin between patients with IBD and controls. P-selectin was the only marker that showed a reduced level in patients with inactive ulcerative colitis.

P-selectin, a member of the cell adhesion molecule family, is produced in cytoplasmic secretory α-granules of platelets and endothelial cells.[18] It is one of the most active factors secreted by endothelial cells, which mediates the activation of leukocytes and adhesion of platelets at the site of vascular injury.[4] A high concentration of soluble P-selectin is a marker of platelet activation, inflammation, and endothelial dysfunction.[10,19] Patients with IBD show an increased soluble P-selectin fraction and expression of P-selectin in the inflamed mucosa.[18-20] However, noninflamed tissues of patients with IBD show decreased levels of P-selectin expression, comparable with those seen in healthy controls.[20] Secretion of P-selectin induces leukocyte rolling, followed by leukocyte adhesion and migration to the inflamed mucosa.[4] In our study, the median level of P-selectin was decreased in patients with inactive ulcerative colitis compared with those with the active disease and controls, correlating with numerous inflammatory markers. In contrast, the Crohn disease group showed no differences between the active and inactive subgroups and no correlations were found, except for WBCs. However, Magro et al[21] reported a lower P-selectin level in patients with active and inactive IBD compared with controls. The authors observed a membrane-bound expression of P-selectin molecules on the surface of endothelial cells, so they suggested that low levels of serum selectins in patients with IBD may be explained by a decreased cleavage from the surface of endothelial cells.[21] In addition, this finding indicated persistent leukocyte activation during remission.[21]

Most endothelial cells (aside from bone marrow endothelial cells) produce and express E-selectin (an endothelial leukocyte adhesion molecule) only after activation of the inflammatory process in response to IL-1β and TNF-α.[22] E-selectin is involved in inducing leukocyte rolling on the endothelial surface.[21] In 1997, Cellier et al[23] showed a positive correlation between E-selectin expression in intestinal biopsies and the clinical and endoscopic severity of IBD. In contrast, Gu et al[24] found no difference in E-selectin or P-selectin expression among 40 patients with IBD with and without relapse. No E-selectin expression was observed in the intestinal tissues of patients with IBD remission.[25]

In our study, we found no difference in the serum level of E-selectin in patients with active versus inactive ulcerative colitis. However, there was a trend towards a lower E-selectin level in the inactive Crohn disease group compared with the active one ($P = 0.052$). The E-selectin concentration positively correlated with numerous inflammatory markers such as CRP, WBC, TNF-α, and IL-6 in patients with ulcerative colitis, and with fibrinogen in patients with Crohn disease. However, no correlation with clinical disease activity assessed with CDAI and the Mayo scale was found. These results are in accordance with observations from several studies conducted in smaller groups of patients.[26,27] In a cohort of 40 patients with IBD, Trzeciak-Jędrzejczyk et al[26] found no differences in serum E-selectin levels among ulcerative colitis, Crohn disease, and control groups. Several studies showed an increased serum E-selectin level only in patients with active Crohn disease compared with those with the quiescent disease.[28,29] Interestingly, Magro et al[21] reported a lower E-selectin concentration in patients (145 subjects) with inactive and biologically active (high CRP levels) Crohn disease compared with controls and clinically active groups. In their study, patients with ulcerative colitis (73 subjects) showed no difference in E-selectin concentrations between biologically active, clinically active, inactive, and healthy control groups.[21] The differences between studies are likely due to various characteristics of the study groups.

Similarly, microparticle formation is enhanced during inflammation and under oxidative stress.[17] Fragments of microparticles released from stimulated or apoptotic cells after plasma membrane remodeling[30] are mostly derived from platelets (70% to 90%) but also from leukocytes, erythrocytes, endothelial cells, smooth muscle cells, and cancer cells.[31,32] Microparticles in body fluids may reflect stem cell damage. They contain the antigens of their cell of origin and can transfer these surface molecules to other cell types.[33] Microparticles play a role in hemostatic and inflammatory processes, vascular remodeling and angiogenesis, cell survival, and apoptosis.[30] The cellular origin of circulating microparticles is dependent on the type of disease, its status, and medical treatment.[32] For example, the level of microparticles can be decreased by numerous medications, including statins, fibrates, β-blockers, and infliximab.[30,34] Under physiological conditions, levels of microparticles and circulating endothelial cells are low; however, they increase with age.[34] Production of microparticles can be enhanced by inflammatory conditions. Voudoukis et al[31] reported lower levels of microparticles in patients with inactive IBD compared with controls, yet increased levels of microparticles in patients with active IBD. Similarly, several studies showed elevated levels of microparticles in patients with active Crohn disease[35] or IBD.[36] In our study, only patients with ulcerative colitis showed a tendency towards increased microparticle levels with increasing disease severity. Of note, the use of immunosuppressive therapy was associated with lower levels of serum microparticles in patients with the active disease.

Mesri et al[37] showed that microparticles induced IL-6 and MCP-1 release in healthy volunteers and tissue factor expression by endothelial cells in vitro. Monocyte chemoattractant protein 1 is produced by dendritic cells, macrophages, fibroblasts, and epithelial and endothelial cells after exposure to TNF-α or IL-1.[38] It acts as a chemoattractant for inflammatory cells such as monocytes, lymphocytes, and natural killer cells.[39] Interleukin 1β released from activated platelets induces endothelial MCP-1, leading to enhanced adhesion of neutrophils to the endothelium.[17] Increased levels of MCP-1 have been found in the mucosa of patients with IBD and in the experimental models of colitis.[40] Khan et al[38] reported that mice lacking the MCP-1 gene had less severe macroscopic and histologic changes when subjected to dinitrobenzenesulfonic acid–induced colitis. Furthermore, interferon γ production was reduced. In contrast, the MCP-1$^{+/+}$ mice showed an increased number of colonic enterochromaffin cells, which was not evident in the MCP-1$^{-/-}$ mice.[38] Monocyte chemoattractant protein 1 disturbs differentiation of monocytes into intestinal macrophages, which may result in the persistence of a reactive macrophage population and chronic inflammation.[39] Motomura et al[40] reported that MCP-1 enhances the production of transforming growth factor β, matrix metalloproteinase 3, and the tissue inhibitor of metalloproteinase 1, which contributes to intestinal fibrosis. Although increased MCP-1 serum levels have been found in patients with IBD, this does not always correlate with its intestinal expression.[41] In our study, we observed no correlation between MCP-1 levels and disease activity, and a weak correlation with IL-6 and TNF-α levels was seen only in the ulcerative colitis group.

Fibroblasts, which express CD40, and T lymphocytes, which express CD40L, interact, activate each other, and promote fibrogenesis.[42] The CD40–CD40L axis is involved in leukocyte–endothelium interactions at the site of the inflamed intestinal mucosa.[43] Both molecules are overexpressed in inflamed colon tissues of patients with IBD and in the ileum of patients with Crohn disease.[44] Furthermore, ileal pouch mucosa leukocytes show CD40 and CD40L upregulation.[45] CD40 is expressed by numerous cells, including endothelial cells, and CD40L is primarily expressed by activated T cells and platelets.[18,43,46] The CD40–CD40L system enhances intestinal inflammation and inflammation-induced mucosal angiogenesis.[44] Activated T cells can stimulate cell adhesion molecules and the expression of soluble mediators through intestinal fibroblasts and microvascular endothelial cells, leading to an enhanced recruitment of T cells.[47] Blockade of CD40–CD40L interactions prevents acute and chronic inflammation.[47] Interestingly, Soendergaard et al[48] demonstrated a higher sCD40L level in patients with Crohn disease unresponsive to vedolizumab treatment. Moreover, Danese et al[49] showed a significant decrease of sCD40L due to infliximab therapy in patients with Crohn disease.

Activated platelets release a large amount of sCD40L.[46] We detected no differences in sCD40L levels among the study groups. Only the ulcerative colitis group showed a positive correlation of sCD40L levels with the Mayo scale score, TNF-α levels, and IL-6 levels (yet not with the platelet count). In contrast, Koutroubakis et al[50] demonstrated increased sCD40L levels in patients with Crohn disease compared with healthy controls, whereas no differences were found in ulcerative colitis groups. However, patients with active ulcerative colitis or Crohn disease had higher sCD40L levels compared with those with the inactive disease. In that study, plasma levels of sCD40L were correlated with platelet count ($R = 0.27$, $P = 0.001$), prothrombin fragment 1 + 2 ($R = 0.16$, $P = 0.03$), thrombin–antithrombin III complex ($r = 0.15$, $P = 0.04$), and P-selectin levels ($r = 0.19$, $P = 0.01$). In a cohort of 149 patients with IBD, Danese et al[46] showed higher levels of sCD40L in individuals with active Crohn disease and ulcerative colitis compared with those with the inactive disease, and higher sCD40L levels in patients with disease remission compared with controls.

The discrepancies between the results of the analyzed studies may be due to differences in the indices used for the assessment of disease activity, study designs, and methods applied for the measurement of the investigated markers. Although we excluded patients with non-IBD diseases, which could have an impact on the study results, several confounding factors, such as concomitant medication, smoking status, BMI, and the level of physical activity, may have influenced endothelial dysfunction.[7] Moreover, iron deficiency, a frequently observed condition in patients with IBD, may contribute to platelet overproduction.[50]

Due to complexity of endothelial function, serum biomarkers may represent different aspects of its activity.

Our study revealed that the serum levels of E-selectin, P-selectin, MCP-1, and sCD40L and the activity of microparticles should not be regarded as clinical markers of IBD activity. However, other studies have suggested that these parameters could be potential indicators of several IBD-related conditions, such as an increased thrombotic risk (microparticles),[17,50] fibrosis (MCP-1),[39] or response to medication (sCD40L).[49]

Admittedly, our study had some limitations. First, it included a relatively small number of patients. Second, we did not perform an endoscopic assessment of Crohn disease activity in patients and based the analysis only on CDAI, which poorly correlates with disease activity evaluated in endoscopic and histological examinations and is also not useful in individuals with a history of extensive ileocolonic resections. Third, the ELISA method used to assess microparticle activity is not the gold standard for that measurement. Finally, the study groups differed regarding the type of medications taken. We analyzed their associations with the analyzed parameters. However, a relatively small number of patients in the subgroups may result in lack of significance when calculating differences.

On the other hand, the strengths of this study included strict exclusion criteria, ruling out any influence of non-IBD diseases.

ARTICLE INFORMATION

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CONTRIBUTION STATEMENT DO, DC, and TM conceived the concept of the study and contributed to research design. DC, KK, HP, KS, and DO were involved in data collection. DC, KS, and DO analyzed and interpreted the data. DC, KK, HP, and KS prepared the original draft of the manuscript. All authors edited and approved the final version of the manuscript.

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REFERENCES

1 Ito F, Sono Y, Ito T. Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: oxidative stress in diabetes, atherosclerosis, and chronic inflammation. Antioxidants (Basel). 2019; 8. pii: E72.

2 Małyszko J, Matuszkiewicz-Rowińska J. Endothelium, asymmetric dimethylarginine, and atherosclerosis in chronic kidney disease. Pol Arch Intern Med. 2018; 128: 145-147.

3 Cibor D, Szczeklik K, Mach T, Owczarek D. Levels of tissue factor pathway inhibitor in patients with inflammatory bowel disease. Pol Arch Intern Med. 2019; 129: 253-258.

4 Rajendran P, Rengarajan T, Thangavel J, et al. The vascular endothelium and human diseases. Int J Biol Sci. 2013; 9: 1057-1069.

5 Premer C, Kanelidis AJ, Hare JM, Schulman IH. Rethinking endothelial dysfunction as a crucial target in fighting heart failure. Mayo Clin Proc Innov Qual Outcomes. 2019; 3: 1-13.

6 Szczeklik K, Krzyściak W, Cibor D, et al. Markers of lipid peroxidation and antioxidant status in the serum and saliva of patients with active Crohn disease. Pol Arch Intern Med. 2018; 128; 362-370.

7 Srivastava K, Kumar R. Stress, oxidative injury and disease. Indian J Clin Biochem. 2015; 30: 3-10.

8 Owczarek D, Cibor D, Głowacki MK, et al. Inflammatory bowel disease: epidemiology, pathology and risk factors for hypercoagulability. World J Gastroenterol. 2014; 20: 53-63.

9 Cibor D, Owczarek D, Butenas S, et al. Levels and activities of von Willebrand factor and metalloproteinase with thrombospondin type-1 motif, number 13 in inflammatory bowel diseases. World J Gastroenterol. 2017; 23: 4796-4805.

10 Cibor D, Domagała-Rodacka R, Rodacki T, et al. Endothelial dysfunction in inflammatory bowel diseases: Pathogenesis, assessment and implications. World J Gastroenterol. 2016; 22: 1067-1077.

11 Van Assche G, Dignass A, Panes J, et al; European Crohn's and Colitis Organisation (ECCO). The second European evidence-based consensus on the diagnosis and management of Crohn's disease: Definitions and diagnosis. J Crohns Colitis. 2010; 4: 7-27.

12 Best WR, Becktel JM, Singleton JW, Kern F Jr. Development of a Crohn's activity disease index. National Cooperative Crohn's disease study. Gastroenterology. 1976; 70: 439-444.

13 Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. N Engl J Med. 1987; 317: 1625-1629.

14 Jin K, Luo Z, Zhang B, Pang Z. Biomimetic nanoparticles for inflammation targeting. Acta Pharm Sin B. 2018; 8: 23-33.

15 Caviglia GP, Rosso C, Ribaldone DG, et al. Physiopathology of intestinal barrier and the role of zonulin. Minerva Biotecnol. 2019; 31:.83-92.

16 Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature. 2007; 448: 427-434.

17 Maślanka K. The role of platelets in inflammatory processes. J Transf Med. 2014; 7: 102-109.

18 Matowicka-Karna J. Markers of inflammation, activation of blood platelets and coagulation disorders in inflammatory bowel diseases. Postepy Hig Med Dosw (Online). 2016; 7: 305-312.

19 Polinska B, Matowicka-Karna J, Kemona H. Assessment of the influence of the inflammatory process on the activation of blood platelets and morphological parameters in patients with ulcerative colitis (colitis ulcerosa). Folia Histochem Cytobiol. 2011; 49: 119-124.

20 Schurmann GM, Bishop AE, Facer P, et al. Increased expression of cell adhesion molecule P-selectin in active inflammatory bowel disease. Gut. 1995; 36: 411-418.

21 Magro F, Araujo F, Pereira P, et al. Soluble selectins, sICAM, sVCAM, and angiogenic proteins in different activity groups of patients with inflammatory bowel disease. Dig Dis Sci. 2004; 49: 1265-1274.

22 Farinacci M, Krahn T, Dinh W, et al. Circulating endothelial cells as biomarker for cardiovascular diseases. Res Pract Thromb Haemost. 2018; 3: 49-58.

23 Cellier C, Patey N, Fromont-Hankard G, et al. In situ endothelial cell adhesion molecule expression in ulcerative colitis. E-selectin in-situ expression correlates with clinical, endoscopic and histological activity and outcome. Eur J Gastroenterol Hepatol. 1997; 9: 1197-1203.

24 Gu P, Theiss A, Han J, Feagins LA. Increased cell adhesion molecules, PECAM-1, ICAM-3, or VCAM-1, predict increased risk for flare in patients with quiescent inflammatory bowel disease. J Clin Gastroenterol. 2017; 51: 522-527.

25 Koizumi M, King N, Lobb R, et al. Expression of vascular adhesion molecules in inflammatory bowel disease. Gastroenterology. 1992; 103: 840-847.

26 Trzeciak-Jędrzejczyk A, Makosiej R, Kolejwa M, et al. The role of adhesion molecules in inflammatory bowel disease in children. Assessment of the possible risk of cardiovascular complications. Przegl Gastroenterol. 2017; 12: 181-185.

27 Vainerc B, Nielsen OH. Serum concentration and chemotactic activity of E-selectin (CD62E) in inflammatory bowel disease. Mediators Inflamm. 1994; 3: 215-218.

28 Adamska I, Czerwionka-Szaflarska M, Kulwas A, et al. Value of E-selectin and L-selectin determination in children and youth with inflammatory bowel disease. Med Wieku Rozwoj. 2007; 11: 413-418.

29 Goggins MG, Goh J, O'Connell MA, et al. Soluble adhesion molecules in inflammatory bowel disease. Ir J Med Sci. 2001; 170: 107-111.

30 Su Y, Chen J, Dong Z, et al. Procoagulant activity of blood and endothelial cells via phosphatidylserine exposure and microparticle delivery in patients with diabetic retinopathy. Cell Physiol Biochem. 2018; 45: 2411-2420.

31 Voudoukis E, Vetsika EK, Giannakopoulou K, et al. Distinct features of circulating microparticles and their relationship with disease activity in inflammatory bowel disease. Ann Gastroenterol. 2016; 29: 180-187.

32 Nomura S, Shimizu M. Clinical significance of procoagulant microparticles. J Intensive Care. 2015; 3: 2.

33 Geddings JE, Mackman N. New players in haemostasis and thrombosis. Thromb Haemost. 2014; 111: 570-574.

34 Morel O, Toti F, Hugel B, et al. Procoagulant microparticles: disrupting the vascular homeostasis equation? Arterioscler Thromb Vasc Biol. 2006; 26: 2594-2604.

35 Leonetti D, Reimund, JM, Tesse A, et al. Circulating microparticles from Crohn's disease patients cause endothelial and vascular dysfunctions. PLoS One. 2013; 8: e73088.

36 Andoh A, Tsujikawa T, Hata K, et al. Elevated circulating platelet-derived microparticles in patients with active inflammatory bowel disease. Am J Gastroenterol. 2005; 100: 2042-2048.

37 Mesri M, Altieri DC. Endothelial cell activation by leukocyte microparticles. J Immunol. 1998; 161: 4382-4387.

38 Khan WI, Motomura Y, Wang H, et al. Critical role of MCP-1 in the pathogenesis of experimental colitis in the context of immune and enterochromaffin cells. Am J Physiol Gastrointest Liver Physiol. 2006; 291: 803-811.

39 Spoettl T, Hausmann M, Herlyn M, et al. Monocyte chemoattractant protein-1 (MCP-1) inhibits the intestinal-like differentiation of monocytes. Clin Exp Immunol. 2006; 145: 190-199.

40 Motomura Y, Khan WI, El-Sharkawy RT, et al. Induction of a fibrogenic response in mouse colon by overexpression of monocyte chemoattractant protein 1. Gut. 2006; 55: 662-670.

41 Singh UP, Singh NP, Murphy EA, et al. Chemokine and cytokine levels in inflammatory bowel disease patients. Cytokine. 2016; 77: 44-49.

42 Sempowski GD, Chess PR, Phipps RP. CD40 is a functional activation antigen and B7-independent T cell costimulatory molecule on normal human lung fibroblasts. J Immunol. 1997; 158: 4670-4677.

43 Danese S, Sans M, Fiocchi C. The CD40/CD40L costimulatory pathway in inflammatory bowel disease. Gut. 2004; 53: 1035-1043.

44 Danese S, Scaldaferri F, Vetrano S, et al. Critical role of the CD40-CD40-ligand pathway in regulating mucosal inflammation-driven angiogenesis in inflammatory bowel disease. Gut. 2007; 56: 1248-1256.

45 Polese L, Angriman I, Giuseppe DF, et al. Persistence of high CD40 and CD40L expression after restorative proctocolectomy for ulcerative colitis. World J Gastroenterol. 2005; 11: 5303-5308.

46 Danese S. Katz JA, Saibeni S, et al. Activated platelets are the source of elevated levels of soluble CD40 ligand in the circulation of inflammatory bowel disease patients. Gut. 2003; 52: 1435-1441.

47 Vogel JD, West GA, Danese S, et al. CD40-mediated immune-nonimmune cell interactions induce mucosal fibroblast chemokines leading to T-cell transmigration. Gastroenterology. 2004; 126: 63-80.

48 Soendergaard C, Seidelin JB, Steenholdt C, Nielsen OH. Putative biomarkers of vedolizumab resistance and underlying inflammatory pathways involved in IBD. BMJ Open Gastroenterol. 2018; 31: 5, e000208.

49 Danese S, Sans M, Scaldaferri F, et al. TNF-alpha blockade down-regulates the CD40/CD40L pathway in the mucosal microcirculation: a novel anti-inflammatory mechanism of infliximab in Crohn's disease. J Immunol. 2006; 176: 2617-24.

50 Koutroubakis IE, Theodoropoulou A, Xidakis C, et al. Association between enhanced soluble CD40 ligand and prothrombotic state in inflammatory bowel disease. Eur J Gastroenterol Hepatol. 2004; 16: 1147-1152.